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(54) Title: ANTIBODIES FOR USE IN TARGETED AND TEMPORARY TREATMENT OF HUMANS AND ANIMALS		
(57) Abstract <p>The invention relates to an antibody, or a fragment of an antibody, which binds to an epitope under specifically chosen conditions, and the binding of which to that epitope is broken under specifically chosen different conditions, in addition to an antibody or fragment thereof with which a diagnostically, therapeutically or cosmetically active substance is optionally conjugated, for targeted and temporary diagnostic, therapeutic or cosmetic treatment of externally accessible parts of the human and animal body, wherein both the specifically chosen binding conditions and the conditions wherein the binding is broken lie within physiologically acceptable limits and are formed by the pH and/or ion strength. The invention further relates to the use of such an antibody or fragment thereof for manufacturing a medication for targeted and temporary treatment of externally accessible parts of the human and animal body, in addition to a composition comprising at least one antibody, or a fragment thereof, according to the invention such as a teeth cleaning agent, mouthwash, mouth spray, chewing tablet, cream or ointment.</p>		

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**ANTIBODIES FOR USE IN TARGETED AND TEMPORARY
TREATMENT OF HUMANS AND ANIMALS**

The invention relates to antibodies or fragments thereof which can be used for "targeting" of (diagnostically, therapeutically and cosmetically) active substances to desired epitopes. The invention further
5 relates to the use of these antibodies or fragments thereof in the targeted and temporary diagnostic, therapeutic and cosmetic treatment of externally accessible parts of the human and animal body.

The use of an antibody-antigen binding to
10 localize for instance a therapeutically or diagnostically active substance on a determined epitope at a specific site of the body is already known per se. Described for instance in US-5490988 and WO 95/01155 are antibodies and fragments of antibodies to which a therapeutically active
15 substance is coupled for the "targeting" of antimicrobial substances to epitopes of pathogenic compounds in the oral cavity. In EP 0736544 are described antibodies which recognise specific protein structures on the tooth, whereby therapeutic or cosmetic substances can be applied
20 specifically and locally to the tooth. Targeted and local administering of active substances achieves maximum effectiveness of these substances.

Through conjugation with a dye (for instance erythrocin) such antibodies or fragments thereof can be
25 used for the detection of for instance dental plaque. Early detection of plaque provides the possibility of removing the plaque before it causes infectious or carious disorders of the teeth and/or surrounding tissues. By bringing the teeth and surrounding tissues
30 into contact with the antibody coupled to a dye which detects the plaque, the plaque can be made visible and then be removed by cleaning the teeth and the surrounding tissues, in particular at those locations where the presence of plaque has been determined.

A drawback of the use of antibodies or fragments thereof for detecting dental plaque via this method is the fact that the antibody/epitope binding is not easily broken, whereby dyes visible to the eye remain
5 visible for a longer time. This can be avoided by using dyes based on fluoresceine which are only visible during irradiation with UV-light. These agents are however rather time-consuming in use and not easy for the consumer to use.

10 The therapeutic treatment of infections in the oral cavity with for instance systemic antibiotics has the important drawback that the antibiotic spreads through the entire body and also reaches other organs and tissues, whereby the natural microbial flora of for
15 instance the gastrointestinal tract can be disrupted. The use of antibiotic gel in the oral cavity may partially prevent this, but has the drawback that it does not remain properly adhered.

It is the object of the invention to obviate
20 the above stated drawbacks. This is achieved by the invention by providing antibodies or fragments thereof which bind to an epitope under specifically chosen conditions, and which elute from that epitope under specifically chosen different conditions. By changing the
25 conditions such that the binding between epitope and antibody is broken, the antibodies can be removed at any desired moment. In this way an infection in for instance the oral cavity can be treated very locally and in targeted manner, and antibodies which are coupled to a
30 dye and which are used for the detection of dental plaque or other oral pathogens can be removed at any desired moment, without lips, tongue and gums remaining coloured for a long time.

An antibody according to the invention is
35 selected using the per se known 'phage-display' technique. Phage-display is a technique for making human antibodies or antibody fragments which can serve for diagnostic or therapeutic purposes and/or for use in

research. The phage-display technique makes use of the possibility of expressing proteins in the coat of bacteriophages. In this technique bacteriophages are transfected with human variable (V) genes or with a combination of human variable and constant genes which form the basis for the composition of human antibodies. By ensuring that the synthesized antibodies are expressed as fusion proteins with a bacteriophagous coat protein (for instance g3p), g3p-antibody fusion proteins appear after synthesis on the surface of the bacteriophage.

There are large so-called phage libraries (or banks) of Fab fragments or of single chain Fv fragments (scFv) with different antibody specificities. Such phage libraries are used in the present invention to select the antibody fragments with the specific property that these antibody fragments bind to a determined epitope under specifically chosen conditions and that the binding between antibody fragment and epitope is broken in specifically chosen different conditions.

These specifically chosen conditions can for instance be formed by the pH or the ion strength. For application in the treatment of the human and animal body these conditions preferably lie within physiologically acceptable limits. For use in the oral cavity the pH can for instance be varied between 4 and 8.5 and the ion strength between 0 and 13 M.

Using a selection procedure as described in Example 1, monoclonal phage-antibody fragments (Fab) are obtained with the properties desired for the application such as affinity, specificity and pH and/or ion strength sensitivity. By optionally changing the expression system it is ultimately possible to bring about that Fab fragments or even intact human monoclonal antibodies are secreted into the culture medium. These antibody fragments or intact antibodies can then be isolated easily from the culture medium.

"Fragments" are understood to mean F(ab), F(ab)', F(ab)'₂ or scFv fragments.

Diagnostically, therapeutically or cosmetically active substances can be coupled to the obtained antibodies or fragments as is described in EP 0453097, EP 0451972 and EP 0450800, or as according to Lal et al.

5 (Immunol. Meth. 1985; 79:307-318).

Such substances can for instance be enzymes, dyes, fluorescent substances, radioactive substances or antimicrobial compounds. Enzymes can be used as antimicrobial agents (therapeutic), or as a bleaching agent for removing deposits on the teeth (cosmetic treatment). Dyes can be used to detect for instance dental plaque or other oral pathogenic micro-organisms (diagnostic). Antimicrobial compounds can be used to treat infections in the oral cavity or other externally accessible parts of the body.

Antibodies or fragments thereof according to the invention can be used for the treatment of externally accessible parts of the human body, such as the oral cavity, but also the skin and the gastro-intestinal and urogenital system.

An example of an application of antibodies according to the invention is bringing antimicrobial compounds into contact with species of the oral microflora. The oral microflora contains a wide diversity of microbial species including the pathogens: Actinomyces actinomycetem comitans, Porphyromonas gingivalis, Prevotella intermedia, Streptococcus mutans, Bacteroides forsythus, Eikenella corrodens, Treponema denticola, Campylobacter lectus, Fusobacterium nucleatum. A number of these species makes a significant contribution toward the forming of plaque. By coupling antimicrobial substances to antibodies or fragments thereof which are targeted against epitopes on these pathogenic compounds, these species can be "attacked" very locally and in targeted manner. As antimicrobial compound can for instance be used an enzyme such as an oxidase (for instance glucose oxidase, lactase oxidase or uric acid oxidase), a peroxidase (for instance horseradish

peroxidase, myeloperoxidase, lactoperoxidase or chloroperoxidase), a protease (for instance papain, pepsin, trypsin, ficin or bromelin), a cell wall-lysing enzyme (for instance lysozyme), or a plaque matrix inhibitor (for instance dextranase or mutanase).

The invention further relates to the use of such antibodies or antibody fragments for the manufacture of a medication for targeted and temporary diagnostic, therapeutic and cosmetic treatment of externally accessible parts of the human and animal body. A medication comprising the antibodies or fragments thereof according to the invention can take a number of forms, depending on the area of the body where the medication has to be applied. For application in the oral cavity the medication can for instance take the form of a mouthwash, a lozenge or chewing tablet, a teeth cleaning agent, cream, ointment or chewing gum.

The invention is further illustrated using the annexed examples and figures. Example 1 describes the selection of antibodies and fragments thereof, wherein a Fab-phage library is used as the starting point. A Fab-phage library can for instance be made as described in Marks et al. (J. Mol. Biol. 222: 581-597, 1991) and as described in Haard et al. (J. Biol. Chem. 274:18218-18230, 1999). The annexed examples and figures serve only for the purpose of illustration and are in no way limiting for the invention.

Figures 1-4 show the DNA patterns of different clones from respective selection procedures A, B, C and D. For this purpose DNA from the clones from the different selection procedures was replicated using PCR, cleaved with restriction-enzyme BstN-1 and subsequently analysed on 3% agarose gel.

EXAMPLES**EXAMPLE 1****Selection of antibodies**

5 The monoclonal antibodies which recognize a specific epitope on the bacterium Staphylococcus epidermidis (S.epidermidis) and which bind reversibly under conditions which can be used in the oral cavity were obtained using a human Fab phage library.

10

1.1 Production of phage particles from a library of antibodies cloned in phagemids in E. coli

 The E. coli bacteria were cultured to an OD_{600nm} of 0.5 (=2*10⁵ bacteria) and helper phages were then added
15 to bring about the formation of phage particles. The infected bacteria were centrifuged and resuspended in culture buffer. After an overnight culture the bacteria were removed using centrifugation, and the phages were precipitated out of the culture medium by means of PEG
20 precipitation. The precipitated phages were resuspended in one of the four binding buffers of table 1, and were subsequently used in the different selection procedures.

1.2 Selection of the phages

25 1*10⁹ bacteria (S.epidermidis) (in the different binding buffers, see table 1) were incubated with phages (input) (see 1.1). The bacteria were then washed 12 times with binding buffer and the phages that had bound were eluted with the correct elution buffer (see table 1).
30 After elution 55% of the bacteria was still alive.

 The eluted phages were replicated via infection of E. coli, followed by culture of the infected E. coli as according to 1.1. The cultured eluted phages were then reused in the following selection round (input). The
35 described selection procedures were carried out 4 times.

Table 1

Selection	Binding buffer	Elution buffer
A	PBS (pH 7.4)	pH 4.5
B	PBS (pH 7.4)	pH 4.5 + 1 M NaCl
C	pH 8.5 + 1 M NaCl	Milli Q
D	pH 8.5	pH 4.5 + 1 M NaCl

Table 2 shows the titration results of the different selection procedures on S.epidermidis. These results demonstrate that for selection procedure B and C the number of specific phages does not increase any further after three selection rounds. In addition, the fact that in selection B, C, and D the titer of the final washing step is lower than the titer of the eluted fraction (output) shows that specific elution has occurred. In selection A this is not the case and there is therefore probably no specific elution in this case. The increasing Output/Input ratio shows the enrichment of binding and eluting phages after the different selection rounds.

1.3 Screening using "whole Cell" ELISA

188 clones (24 clones from round 3 and 23 clones from round 4 of selection A and D, and 42 clones from round 3 and 5 clones from round 4 of selection B and C) were selected at random and tested as phage-antibodies in the Whole Cell ELISA in a 96-well plate as described below, for screening for antibodies which bind specifically to S.epidermidis.

Table 2

Selection	Round	Input ¹	Titer ²	Output ³	Output/Input
A	1	6.9×10^{12}	-	7.5×10^4	1.1×10^{-8}
	2	7.4×10^{13}	-	1.7×10^5	2.3×10^{-9}
	3	1.8×10^{13}	1.1×10^8	1.3×10^7	7.2×10^{-7}
	4	2.5×10^{13}	8.8×10^8	2.0×10^8	8.0×10^{-6}
B	1	5.4×10^{12}	-	5.8×10^5	1.1×10^{-7}
	2	8.3×10^{13}	-	6.6×10^7	8.0×10^{-7}
	3	8.3×10^{12}	5.5×10^9	3.4×10^{10}	4.1×10^{-3}
	4	5.8×10^{12}	6.5×10^9	3.9×10^{10}	6.7×10^{-3}
C	1	6.0×10^{12}	-	1.2×10^6	2.0×10^{-7}
	2	2.7×10^{13}	-	2.0×10^7	7.4×10^{-7}
	3	1.1×10^{13}	3.1×10^9	3.4×10^{10}	3.1×10^{-3}
	4	4.7×10^{12}	2.1×10^9	3.1×10^{10}	6.6×10^{-3}
D	1	6.5×10^{12}	-	1.9×10^7	2.9×10^{-6}
	2	3.6×10^{13}	-	1.1×10^8	3.1×10^{-6}
	3	1.9×10^{13}	1.7×10^8	5.0×10^9	2.6×10^{-4}
	4	9.3×10^{12}	4.9×10^8	1.1×10^{10}	1.2×10^{-3}

¹ Input (total cfu) (cfu=colony forming unit)

² Titer (total cfu of the final washing step before elution)

³ Output (total cfu)

Monoclonal phage particles (individual clones) were incubated with S.epidermidis (2×10^7 bacteria per well) in binding buffer in a 96-well plate. The bacteria were then washed thoroughly with binding buffer (by means of centrifuging followed by resuspension), whereafter the phage particles were eluted with elution buffer (or binding buffer as control) for 30 minutes. After the

elution the bacteria were centrifuged and the presence of phage particles on the bacteria was demonstrated by means of peroxidase-labelled mouse-anti-M13 antibodies and 3,3',5,5'-tetramethylbenzidine (TMB). The plate was
5 measured at 405nm. In the Whole Cell ELISA the same combinations of binding and elution buffers were used as during the selection.

Clones with an ELISA signal which was twice as high as the background signal when elution took place
10 with the binding buffer (control) were designated "positive clones". Positive clones are therefore all clones which bind specifically to the bacterium at the chosen binding condition, irrespective of the fact of whether they elute in other conditions. Clones with an
15 ELISA signal after elution with binding buffer which was twice as high as the ELISA signal after elution with elution buffer were designated "specifically eluting clones". The specifically eluting clones are therefore the antibody-phage clones which bind in the binding
20 buffer used, and which do not elute in the binding buffer but do so when washed with the elution buffer. The specifically eluting clones therefore bind under specifically chosen conditions and elute under specifically chosen different conditions. Table 3 shows
25 the results of the Whole Cell ELISA.

The fact that in the group of clones from selection A not a single specifically eluting clone was found confirms the supposition that in selection A specific elution did not occur (see 1.2).

Table 3

Selection	number of positive clones	number of specifically eluting clones
A	47/47	0/47
B	47/47	30/47
C	47/47	7/47
D	25/47	13/47

1.4 "Fingerprint analysis" of the positive clones

All 188 clones from the 4 selections were screened with fingerprint analysis as described hereinbelow in order to determine the diversity of the clones. Using the fingerprint analysis DNA patterns were obtained which were subsequently used to distinguish the different clones. The clones were tested for the presence of an insert of 1700 bp (clones which code for Fab must have an insert of about 1700 bp). All tested clones (except 1 clone from selection C, and 8 clones from selection D) contained the specific insert.

For the fingerprint analysis, DNA of the individual clones was replicated using PCR and then cleaved with restriction-enzyme BstN-1. The DNA samples were analysed on 3% agarose gel with 0.5 µg/ml ethidium bromide. Shown in figures 1-4 are the DNA patterns of the individual clones. The clones are numbered in accordance with their position on the ELISA plate. The results show that respectively 2, 12, 13 and 32 different DNA patterns ("fingerprints") are present in selection A, B, C and D. Patterns 4, 5 and 14 occur in both selection B and D. Patterns 10 and 12 are found in both selection B and C. Pattern 24 occurs in selection C and D and pattern 3 is found in selections B, C and D. There are therefore clones which elute due to a pH-shock, and there are other clones which elute due to a salt-shock, or through a combination of pH and salt-shock.

When the results of the Whole Cell ELISA are compared to the results of the DNA patterns (see table

4), there are 16 clones (with different DNA patterns) which are positive in the binding to S.epidermidis and which elute specifically after binding.

5 Table 4

	fingerprint pattern	selection
	6, 7, 8, 9, 11, 13	B
	17, 18, 21, 22	C
	47, 51	D
10	12	B, C
	5, 14	B, D
	3	B, C, D

1.5 Determining the specificity

15 The 16 specific clones according to the invention were tested in an ELISA for binding to streptavidin, biotin-labelled BSA, thyroglobulin and to an empty well. None of the 16 clones bound to any of the coated antigens. The binding to S.epidermidis in elution
20 buffer and to S.epidermidis in binding buffer was tested in the Whole Cell ELISA. All 16 clones according to the invention bound only to S.epidermidis in binding buffer and not in elution buffer.

25 **EXAMPLE 2**

Toothpaste

A clone from selection C (Example 1) was used to prepare a toothpaste with phage-antibody fragments coupled to erythrocin for the purpose of demonstrating
30 the presence of S.epidermidis in the oral cavity. The phage-antibody fragments from selection procedure C bind to S.epidermidis at a pH of 8.5 and a salt concentration of 1 M NaCl. The binding of antibody to S.epidermidis is made visible by the bound dye. By then rinsing with water
35 (pH 7) the binding is broken and the bound erythrocin removed.

EXAMPLE 3**Ion strength as specifically chosen condition**

An ion strength of between about 0 and 13 M can be used in the oral cavity.

5 In a different embodiment according to the invention the antibody or a fragment thereof having coupled thereto a suitable diagnostically, therapeutically or cosmetically active substance binds at an ion strength of between 0 and 500 mM. The binding is
10 then broken at any desired moment by rinsing with a mouthwash having an ion strength of 1-13 M.

CLAIMS

1. Antibody, or a fragment of an antibody, which binds to an epitope under specifically chosen conditions, and the binding of which to that epitope is broken under specifically chosen different conditions.

5 2. Antibody, or a fragment of an antibody, as claimed in claim 1, with which a diagnostically, therapeutically or cosmetically active substance is conjugated.

10 3. Antibody, or a fragment of an antibody, as claimed in claim 1 or 2, **characterized in that** both the specifically chosen binding conditions and the conditions wherein the binding is broken lie within physiologically acceptable limits.

15 4. Antibody, or a fragment of an antibody, as claimed in claim 3, **characterized in that** the specifically chosen conditions are formed by the pH.

20 5. Antibody, or a fragment of an antibody, as claimed in claim 4, **characterized in that** the antibody binds to an epitope at a pH between 4-8.5 and that the binding is broken at a different pH between 4-8.5.

 6. Antibody, or a fragment of an antibody, as claimed in claim 4, **characterized in that** the antibody binds to an epitope at a pH of about 4-7 or 7-8.5 and that the binding is broken at a pH of about 7.

25 7. Antibody, or a fragment of an antibody, as claimed in claim 4, **characterized in that** the antibody binds to an epitope at a pH of about 7 and that the binding is broken at a pH of about 4-7 or 7-8.5.

30 8. Antibody, or a fragment of an antibody, as claimed in claim 4, **characterized in that** the antibody binds to an epitope at a pH of about 4-6 or 8-8.5 and that the binding is broken at a pH of about 7.

35 9. Antibody, or a fragment of an antibody, as claimed in claim 4, **characterized in that** the antibody binds to an epitope at a pH of about 7 and that the binding is broken at a pH of about 4-6 or 8-8.5.

10. Antibody, or a fragment of an antibody, as claimed in claim 3, **characterized in that** the specifically chosen conditions are determined by the ion strength.

5 11. Antibody, or a fragment of an antibody, as claimed in claim 10, **characterized in that** the antibody binds at an ion strength between 0 and 13 M and that the binding is broken at a different ion strength between 0 and 13 M.

10 12. Antibody, or a fragment of an antibody, as claimed in claim 10, **characterized in that** the antibody binds at an ion strength of about 0-500 mM and that the binding is broken at an ion strength of about 1-13 M.

15 13. Antibody, or a fragment of an antibody, as claimed in claim 3, **characterized in that** the antibody binds to an epitope at a pH between 4-8.5 and/or an ion strength between 0 and 13 M and that the binding is broken at a different pH between 4-8.5 and/or a different ion strength between 0 and 13 M.

20 14. Antibody, or a fragment of an antibody, as claimed in any of the foregoing claims, **characterized in that** the fragment is an F(ab), F(ab)', F(ab)'₂ or an scFv.

25 15. Antibody, or a fragment of an antibody, as claimed in any of the foregoing claims for targeted and temporary diagnostic, therapeutic and cosmetic treatment of externally accessible parts of the human and animal body.

30 16. Antibody, or a fragment of an antibody, as claimed in claim 15, **characterized in that** an externally accessible part of the human or animal body is the oral cavity.

35 17. Antibody, or a fragment of an antibody, as claimed in claim 16, **characterized in that** the cosmetic treatment of the oral cavity comprises the bleaching of teeth and molars.

18. Antibody, or a fragment of an antibody, as claimed in claim 16, **characterized in that** the diagnostic

treatment of the oral cavity comprises the detection of plaque.

19. Antibody, or a fragment of an antibody, as claimed in claim 16, **characterized in that the**
5 therapeutic treatment of the oral cavity comprises the removal of plaque.

20. Antibody, or a fragment of an antibody, as claimed in claim 15, **characterized in that the**
10 therapeutic treatment comprises the treatment of infections in externally accessible parts of the human or animal body.

21. Antibody, or a fragment of an antibody, as claimed in any of the foregoing claims 2-20, wherein the active substance is an enzyme.

15 22. Antibody, or a fragment of an antibody, as claimed in claim 21, **characterized in that** the enzyme is an oxidase (for instance glucose oxidase, lactase oxidase or uric acid oxidase), a peroxidase (for instance horseradish peroxidase, myeloperoxidase, lactoperoxidase
20 or chloroperoxidase), a protease (for instance papain, pepsin, trypsin, ficin or bromelin), a cell wall-lysing enzyme (for instance lysozyme), or a plaque matrix inhibitor (for instance dextranase or mutanase).

23. Antibody, or a fragment of an antibody, as
25 claimed in any of the foregoing claims 2-20, wherein the active substance is a fluorescent or radioactive substance.

24. Antibody, or a fragment of an antibody, as claimed in any of the foregoing claims 1-23,
30 **characterized in that** the antibody, or fragment of an antibody, is targeted against epitopes of pathogenic micro-organisms or other pathogenic compounds.

25. Antibody, or a fragment of an antibody, as claimed in claim 24, **characterized in that** the pathogenic
35 micro-organisms are for instance Actinomyces actinomycetem comitans, Porphyromonas gingivalis, Prevotella intermedia, Streptococcus mutans, Bacteroides

forsythus, Eikenella corrodens, Treponema denticola,
Campylobacter lectus or Fusobacterium nucleatum.

26. Composition comprising at least one
antibody, or a fragment of an antibody, as claimed in any
5 of the foregoing claims 1-25 together with a usual
diluent or solvent or usual carrier.

27. Composition as claimed in claim 26,
characterized in that the composition is a teeth cleaning
agent, mouthwash, mouth spray, chewing tablet, chewing
10 gum, cream or ointment.

28. Use of an antibody, or a fragment of an
antibody, as claimed in any of the foregoing claims 1-25
for the manufacture of a medication for targeted and
temporary treatment of externally accessible parts of the
15 human and animal body.

1/12

M A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 B1 B2 B3 B4 B5 B6 M
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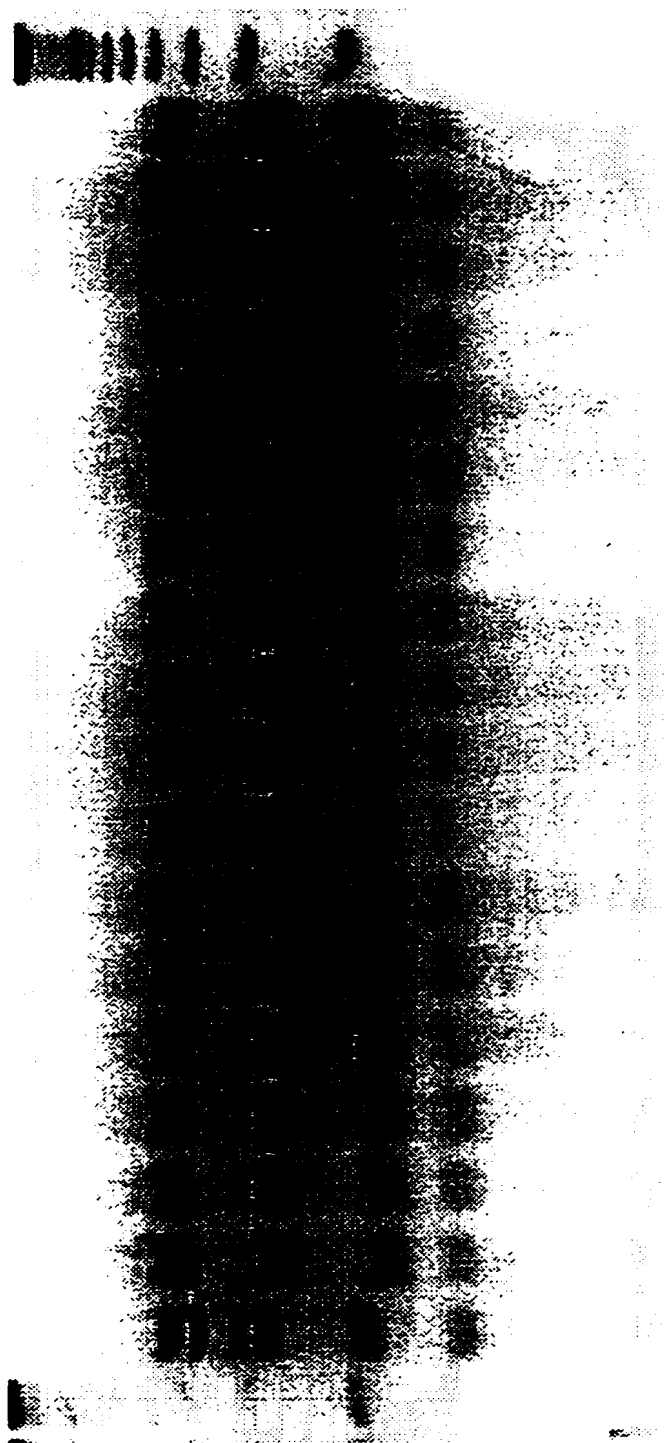


Fig.1a

100
100

2/12

M B7 B8 B9 B10 B11 B12 C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12
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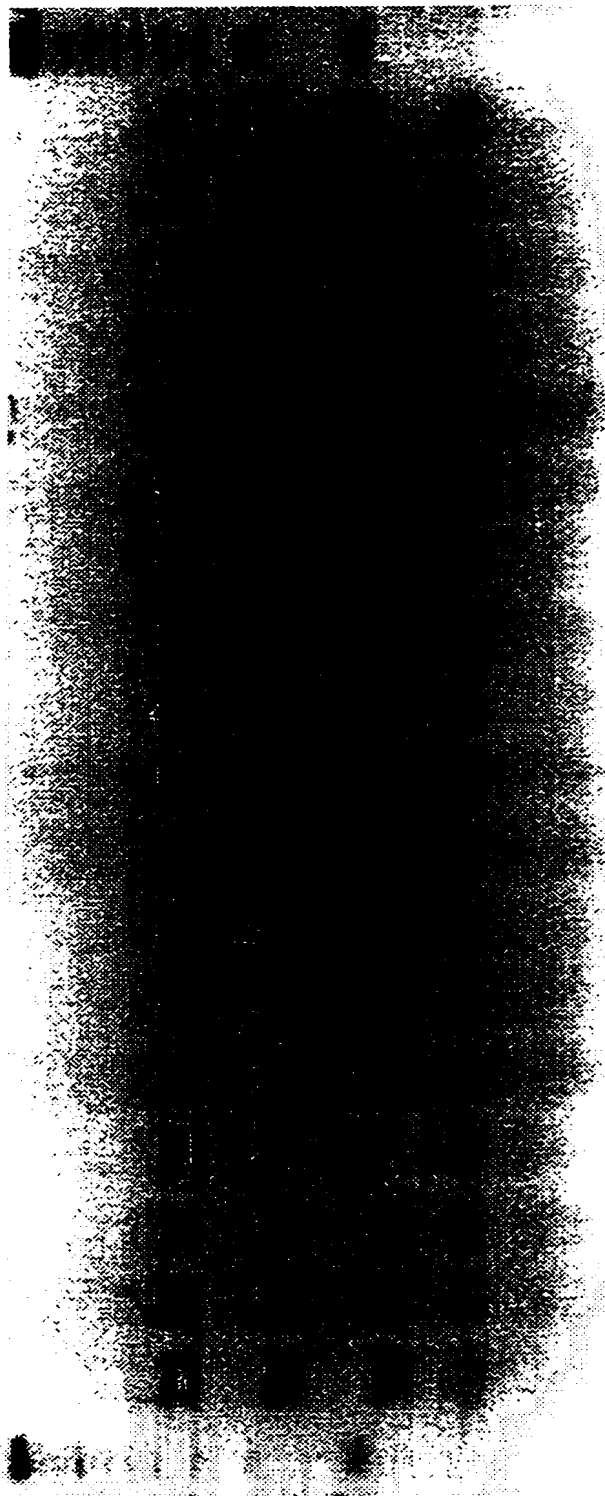


Fig.1b



3/12

M D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12
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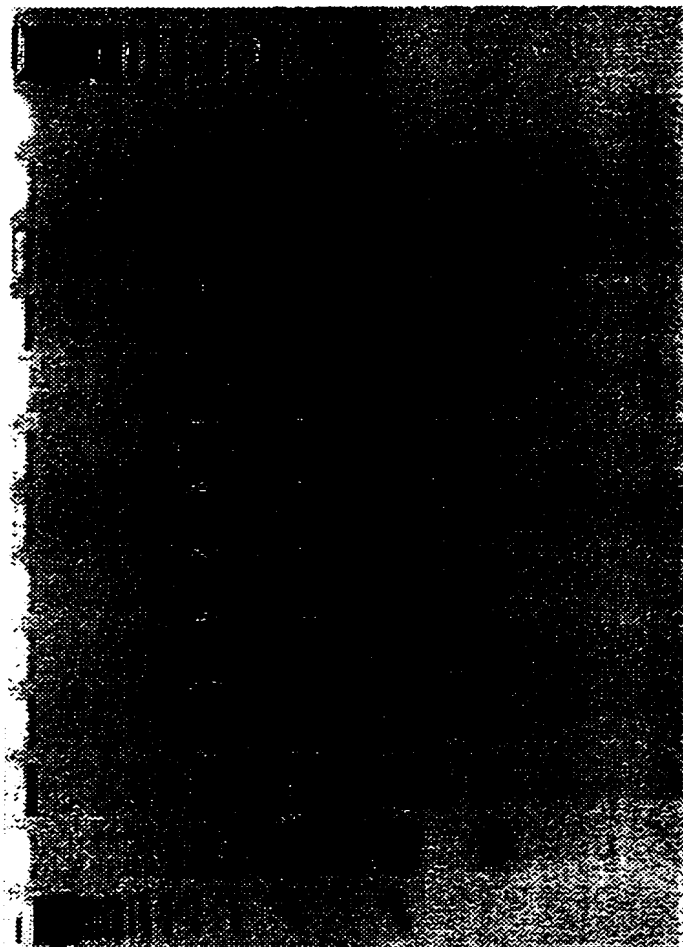


Fig. 1c

The first part of the paper discusses the importance of maintaining accurate records of all transactions. It is essential for the business to have a clear and concise record of all income and expenses, as this will be necessary for the preparation of the tax return. The second part of the paper discusses the importance of keeping up to date with the latest tax laws and regulations. It is important to consult with a tax professional to ensure that the business is compliant with all applicable laws. The third part of the paper discusses the importance of maintaining proper documentation for all transactions. This includes keeping receipts, invoices, and other documents that can be used to verify the accuracy of the records. The fourth part of the paper discusses the importance of having a clear understanding of the business's financial situation. This includes knowing the current balance of the business's bank accounts, as well as the amount of cash on hand. The fifth part of the paper discusses the importance of having a clear understanding of the business's tax obligations. This includes knowing the amount of taxes that must be paid, as well as the deadlines for payment. The sixth part of the paper discusses the importance of having a clear understanding of the business's financial goals. This includes knowing the amount of profit that the business is aiming to achieve, as well as the amount of cash that is needed to maintain the business. The seventh part of the paper discusses the importance of having a clear understanding of the business's financial risks. This includes knowing the amount of cash that is needed to cover the business's operating expenses, as well as the amount of cash that is needed to cover the business's capital expenses. The eighth part of the paper discusses the importance of having a clear understanding of the business's financial opportunities. This includes knowing the amount of cash that is needed to invest in new equipment, as well as the amount of cash that is needed to invest in new personnel. The ninth part of the paper discusses the importance of having a clear understanding of the business's financial challenges. This includes knowing the amount of cash that is needed to cover the business's operating expenses, as well as the amount of cash that is needed to cover the business's capital expenses. The tenth part of the paper discusses the importance of having a clear understanding of the business's financial future. This includes knowing the amount of cash that is needed to cover the business's operating expenses, as well as the amount of cash that is needed to cover the business's capital expenses.

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M A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 B1 B2 B3 B4 B5 B6 M
 (3) (4) (3) (3) (5) (3) (3) (4) (3) (3) (3) (6) (4) (7) (4) (3) (3) (3)

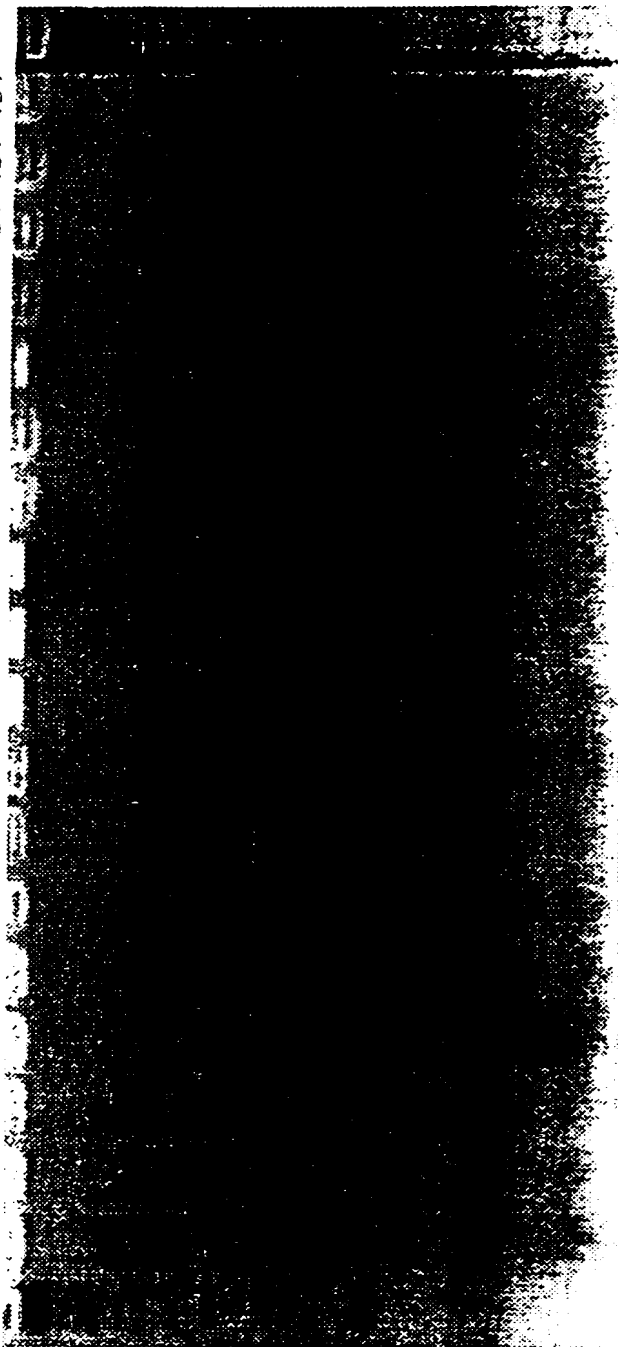


Fig. 2a

5/12

M B7 B8 B9 B10 B11 B12 C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 M
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Fig. 2b

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M D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12
 (3) (12) (13) (3) (3) (3) (3) (14) (3) (3) (3) (-) M

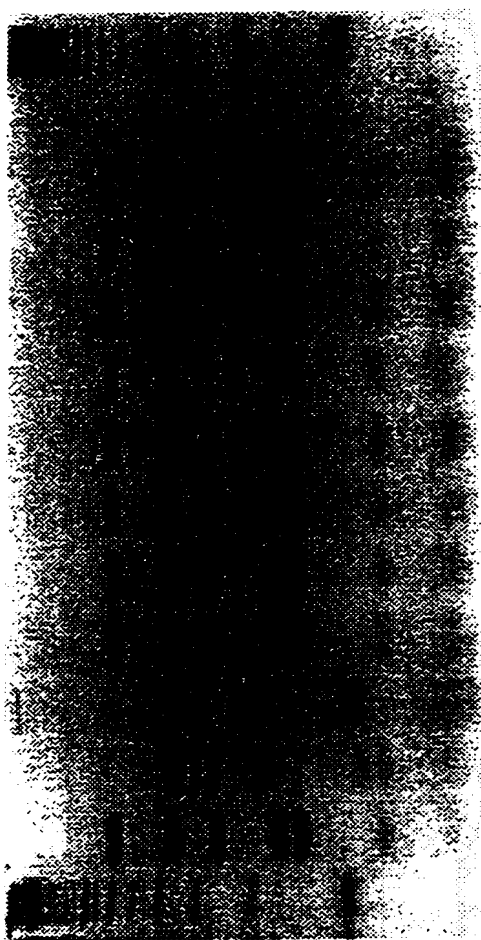


Fig. 2c



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A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 B1 B2 B3 B4 B5 B6
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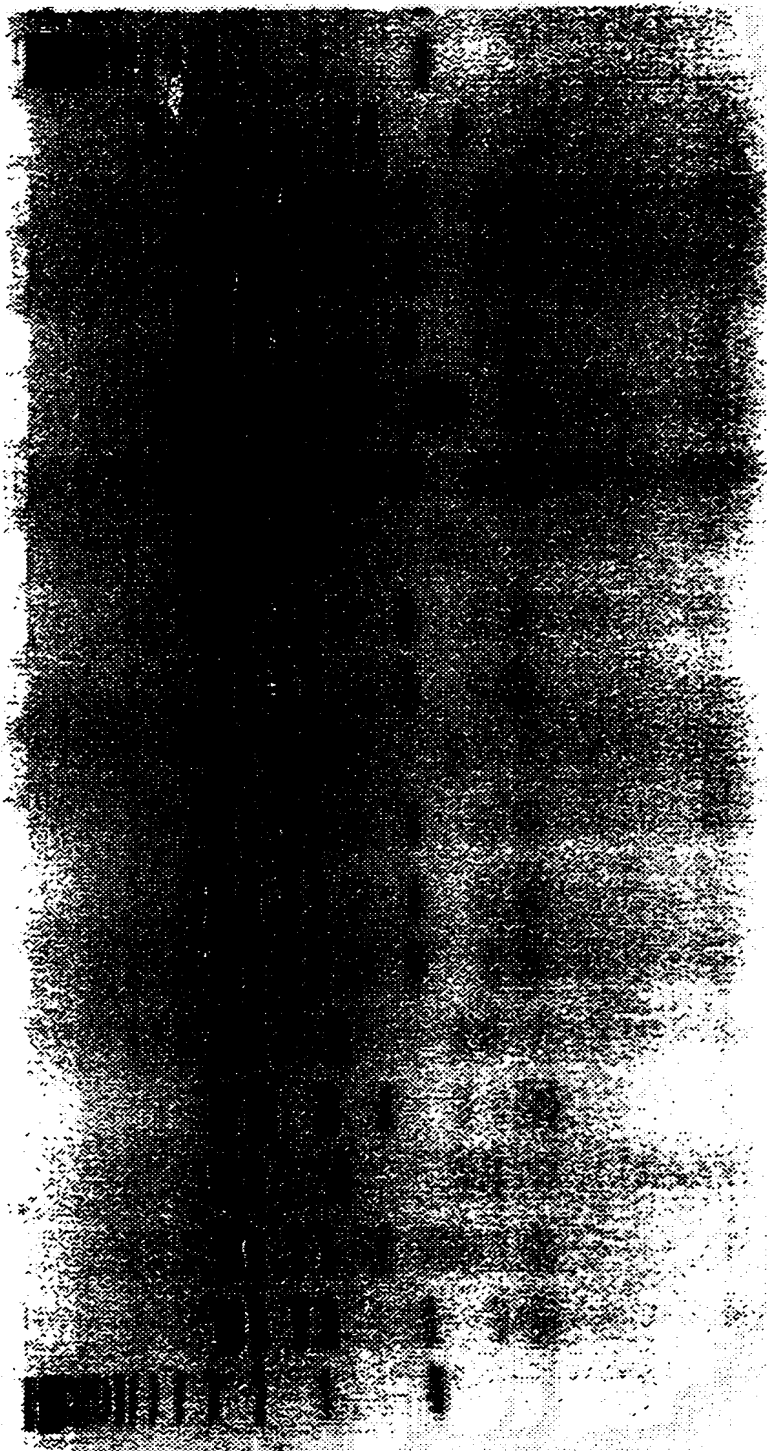


Fig.3a

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for the success of any business and for the protection of the interests of all parties involved. The document then goes on to describe the various methods and techniques used to collect and analyze data, and to provide a detailed account of the results of the study. The final part of the document discusses the implications of the findings and offers suggestions for further research.

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M B7 B8 B9 B10 B11 B12 C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12
(12)(19) (16)(16) (20) (3) (15)(16) (21) (16) (15)(15)(19) (16)(22)(17) (10)(15) M

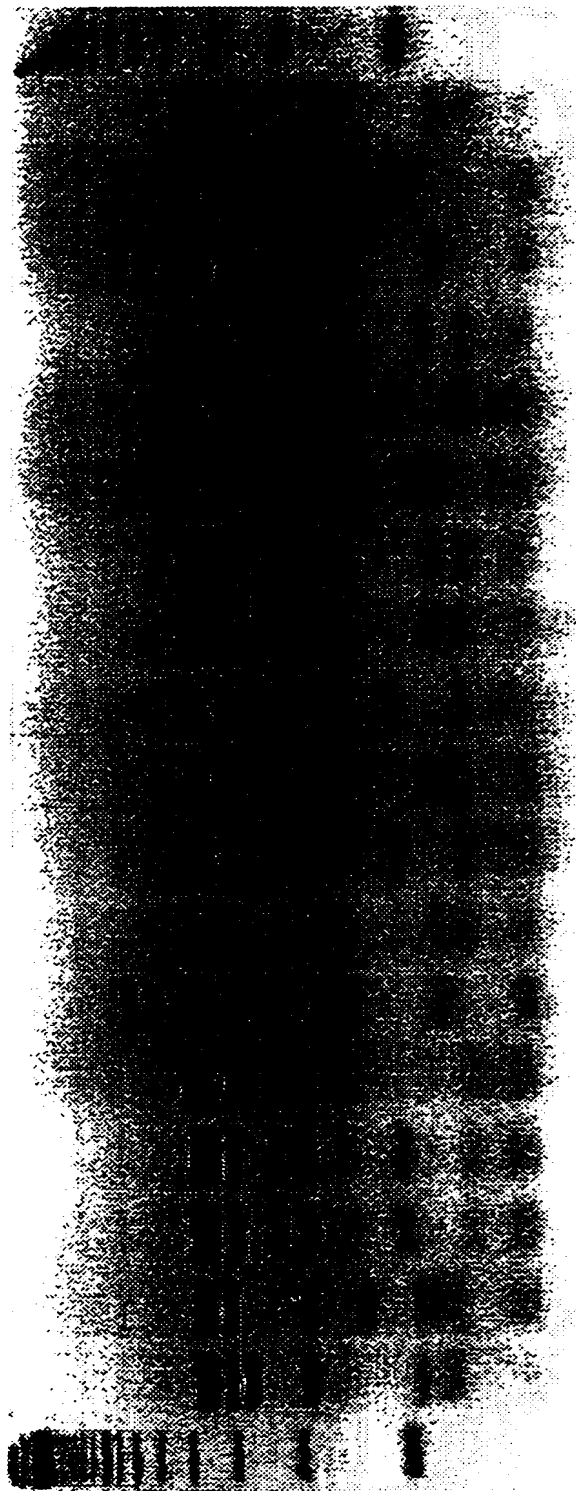
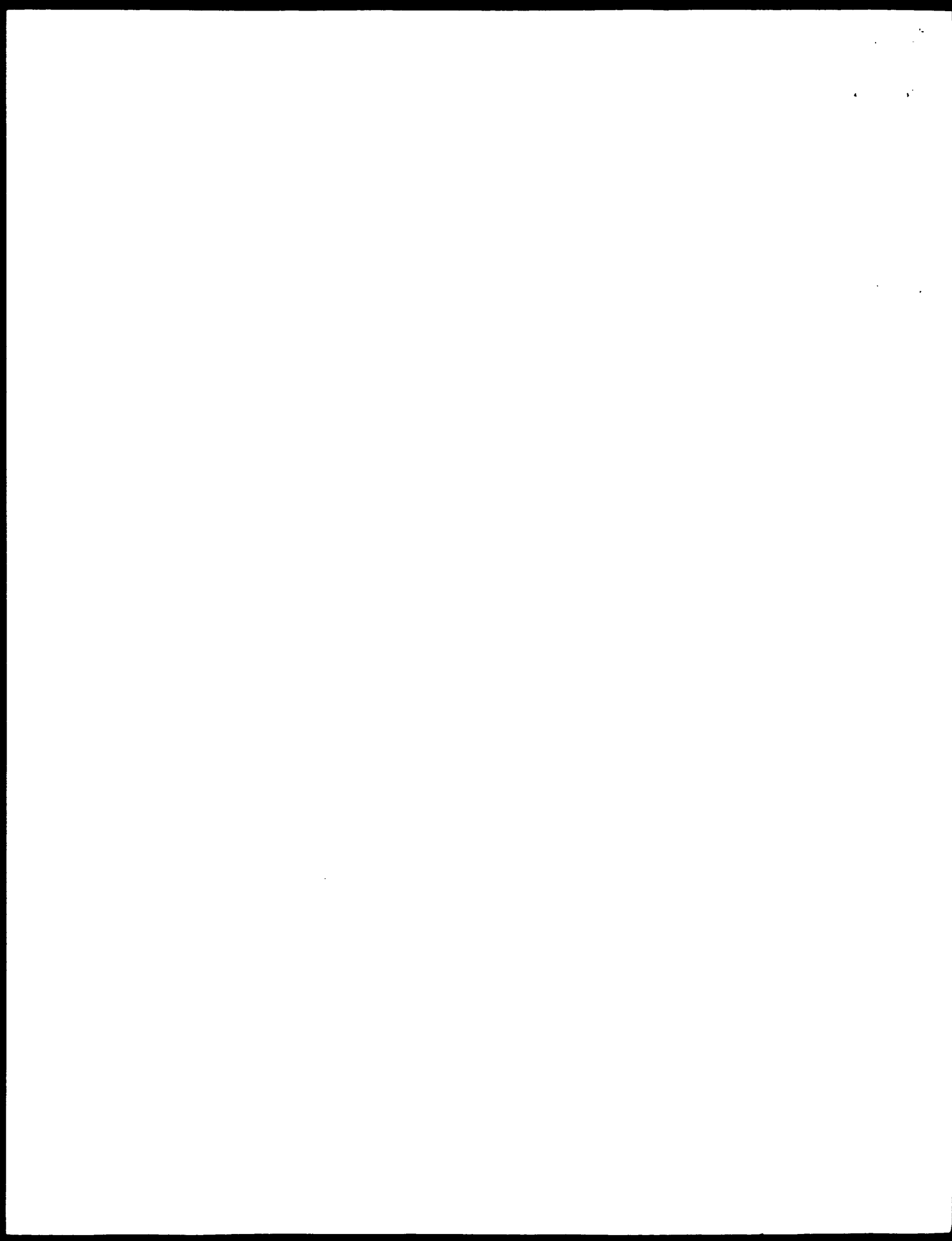


Fig.3b



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M D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12
(3) (12) (13) (3) (3) (3) (3) (14) (3) (3) (3) (-) M

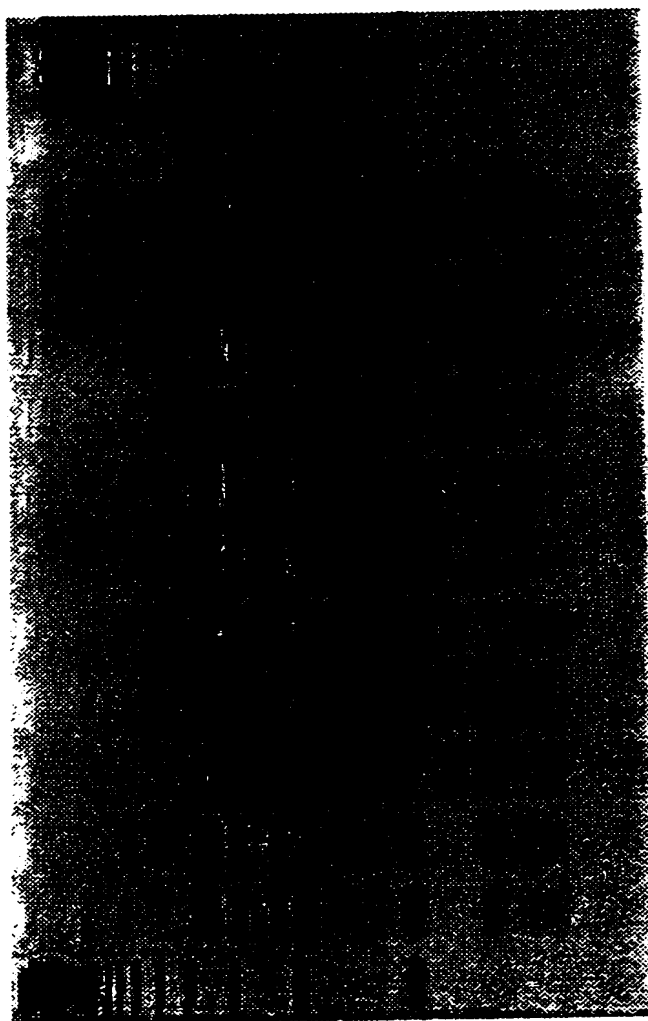


Fig.3c

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M A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 B1 B2 B3 B4 B5 B6
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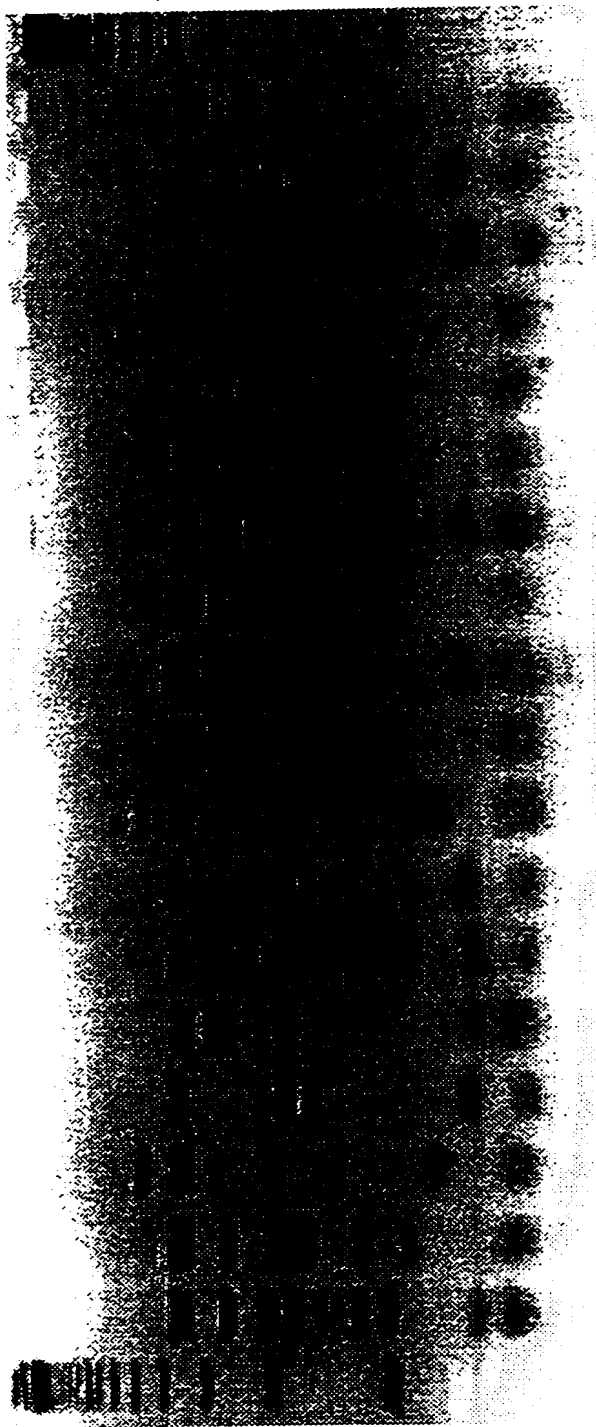


Fig.4a

11/12

B7 B8 B9 B10 B11 B12 C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12
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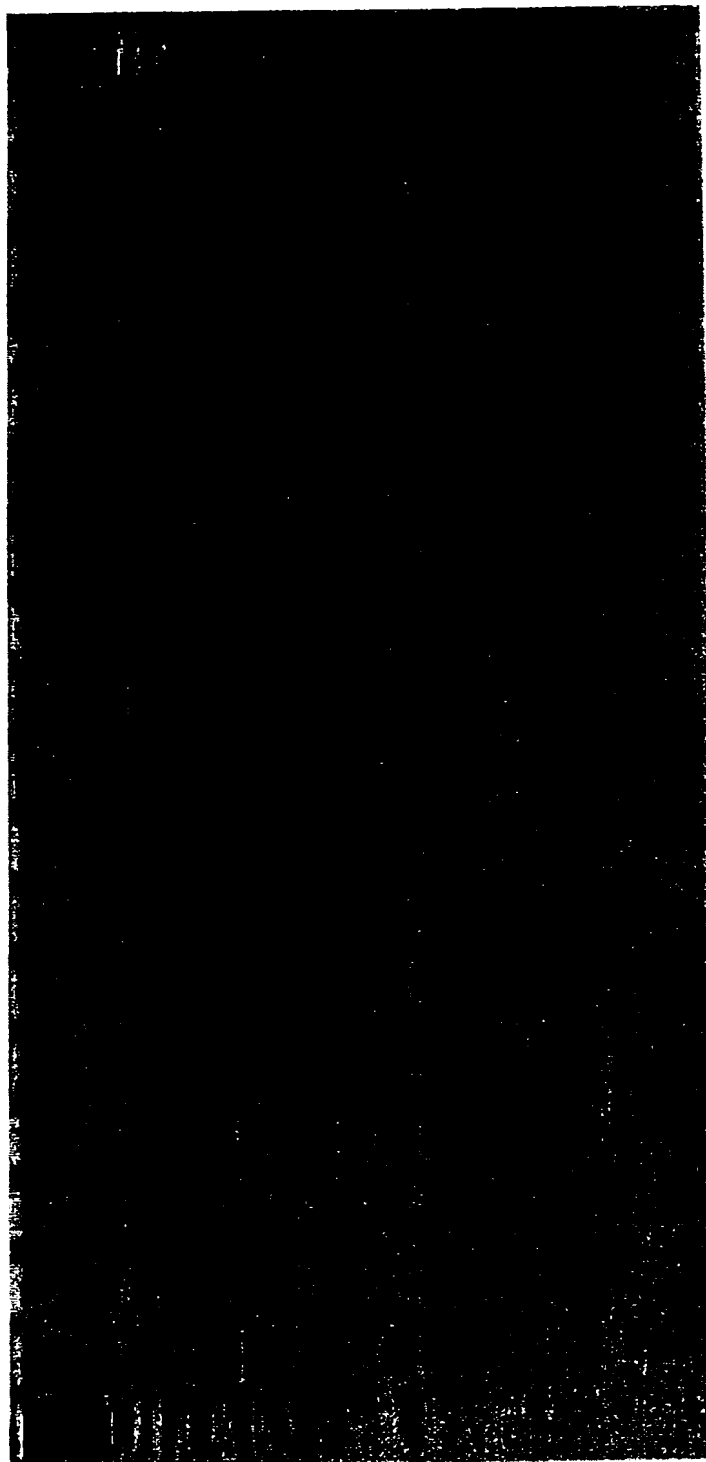


Fig. 4b

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M D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 M
(3) (4) (49) (50) (3) (14) (51) (3) (14) (14) (-) (-)

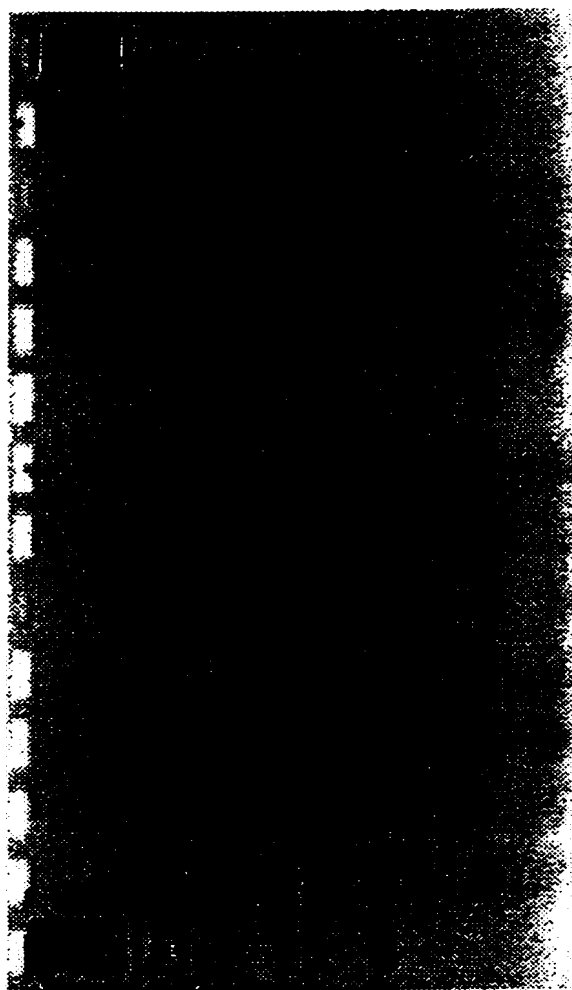


Fig.4c

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/NL 99/00509

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/48 A61K7/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 87 03205 A (CORAL SOCIEDADE BRASILEIRA DE) 4 June 1987 (1987-06-04) claims	1
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

23 November 1999

Date of mailing of the international search report

29/11/1999

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Berte, M

INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/NL 99/00509

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	US 5 490 988 A (VERHOEYEN MARTINE E ET AL) 13 February 1996 (1996-02-13) cited in the application column 6, line 19 - line 22; claims -----	1-28
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